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## Biodegradation of oil sands process affected water in sequencing batch reactors and microbial community analysis by high-throughput pyrosequencing

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#### ABSTRACT

Two sequencing batch reactors (SBR) were constructed and filled with different inocula of activated sludge (AS) and mature fine tailings (MFT) to treat oil sands process-affected water (OSPW). The COD was reduced by 82% in the AS-SBR and 43% in the MFT-SBR during phase I using 10% OSPW and 90% synthetic wastewater as reactor feed. However, COD removal reached 12% and 20% in the AS-SBR and the MFT-SBR, respectively, when 100% raw OSPW was fed into the reactors. Maximum removal of acid-extractable organics (AEO) was 8.7% and 16.6% in the AS-SBR and the MFT-SBR, respectively with a hydraulic retention time of one day. Pyrosequencing analysis revealed that *Proteobacteria* was the dominant phylum and *beta*- and *gamma-Proteobacteria* were dominant classes in both reactors. Evidence of a microbial community change was observed when influent raw OSPW was switched from 50 to 100%. More significant changes in the AS-SBR community were detected.

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### 1. Introduction

Development and growth of the Athabasca oil sands industry in northern Alberta, Canada, has been rapid during the last few decades. The Athabasca oil sands are one of the largest reserves of crude oil in the world, followed by those in Saudi Arabia and Venezuela. Oil production is projected to reach over 3.3 million barrels per day by 2020 (Schindler, 2010) and to generate 300 billion Canadian dollars in tax revenue over the next 25 years (Government of Alberta, 2006). The surface mining procedure typically employs an alkaline hot water extraction process to separate bitumen from the oil sands. Approximately 2–2.5 cubic meters of fresh water is required to produce one cubic meter of crude oil (Toor et al., 2013). The used water is recycled to reduce fresh water withdrawal. Besides a residue of sand and clay it picks up from the oil sands, the recycled OSPW contains high concentrations of organic and inorganic compounds, including naphthenic acids (NAs), benzene, toluene, polycyclic aromatic hydrocarbons, sulfate, and chloride (Hwang et al., 2013) and it must be stored in tailings ponds until the water is safe for environmental release. A limit for the extension of tailings ponds and timelines for OSPW

reclamation have been mandated by the province of Alberta (Ramos-Padrón et al., 2010). Treatment methods that allow extended recycling and safe release of OSPW would decrease the intake of fresh water from the Athabasca River and reduce the environmental threat of the tailings ponds.

OSPW quality can be improved by physical, chemical, and/or biological processes such as coagulation and flocculation (Pourrezaei et al., 2011), adsorption (Gamal El-Din et al., 2011), advanced oxidation (Drzewicz et al., 2010; Garcia-Garcia et al., 2011), membrane filtration (Kim et al., 2011), and bioreactors (Huang et al., 2012; Hwang et al., 2013; Toor et al., 2013). Treatments that employ microorganisms have been found to be environmentally friendly and economical. Reported studies on bioreactor applications for OSPW focus on attached growth systems, i.e., biofilm reactors. Previous study on the removal of three model NAs in circulating packed-bed reactors showed that degradation of NAs was influenced by carbon number and the spatial arrangement of NA alkyl branches (Huang et al., 2012). Bioreactor treatment showed that a biofilm developed in a rotating annular bioreactor using lake water had little impact on the degradation of OSPW NAs (Headley et al., 2010). Hwang et al. (2013), studying OSPW treatment using biofilm reactors, concluded that combined ozonation and biofilm degradation could be used to reduce acidextractable organics (AEO) and NAs in OSPW. Choi et al. (2014) investigated the impact of biofilm reactor configuration (batch vs.

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Fig. 1. Reactor set-up and operating cycle.

continuous flow) on bioreactor performance in treating OSPW. It was reported that continuous flow conditions could significantly improve OSPW treatment and microbial population dynamics. Recently, fluidized bed biofilm reactors were applied for OSPW treatment using granular activated carbon (GAC) as the biofilm support medium. Significantly improved AEO and NAs removal was observed in the reactors, which was attributed to synergy between GAC adsorption and biofilm degradation (Islam et al., 2014). However, limited studies have been performed to evaluate suspended growth systems. Application of suspended growth systems should be evaluated to enable a better understanding of their potential to remediate OSPW in the oil sands industry.

In suspended growth systems, differing biomass inoculations can affect reactor start-up and performance. For example, activated sludge (AS) biomass that contains diverse bacterial communities has been widely used for industrial wastewater treatment (Wang et al., 2007; Monsalvo et al., 2009; Pitás et al., 2012). Inoculation with indigenous bacteria from contaminated sites can also provide bioremediation, leading to bacterial stimulation in industry-contaminated sites (Gallego et al., 2008; Hwang et al., 2013).

This study investigated the performance of sequencing batch reactors (SBRs) inoculated with AS- and MFT biomass for OSPW treatment. SBRs, operated with sequential stages of filling, reaction, settling, decanting, and idling, have been widely used to treat municipal and industrial wastewater (Lyles et al., 2008; Wittebolle et al., 2009; Pendashteh et al., 2010). The comparison of different inocula provides important information on the bioreactor start-up strategies when bioreactors are operated in full-scale within an OSPW treatment facility. High-throughput pyrosequencing techniques were utilized to analyze and compare the microbial communities in each SBR. We believe this is the first report of high-throughput pyrosequencing of microbial communities in SBR treatments of oil sands tailings water.

#### 2. Materials and methods

#### 2.1. Reactor set-up and operation

Sequencing batch reactors (1 L) were inoculated, one with MFT from an oil sands tailings pond in Fort McMurray, Northern Alberta and the other with AS from a local wastewater treatment plant in Edmonton, Alberta. The SBRs were operated using 24-h cycles (Fig. 1) consisting of a 23-h aerobic period in which dissolved oxygen (DO) was maintained at 4–6 mg/L, a 30-min settling period,

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